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Short communication

Synthesis of some novel 2-substituted-5-[isopropylthiazole] clubbed 1,2,4-triazole and 1,3,4-oxadiazoles as potential antimicrobial and antitubercular agents

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Clubbed triazole-thiazole derivatives
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ABSTRACT

In the present study a series of 2-substituted-5-[isopropylthiazole] clubbed 1,2,4-triazole and 1,3,4-oxadiazole derivatives have been synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. Synthesized compounds were evaluated for their preliminary cytotoxicity, antimicrobial and antitubercular activity against *Mycobacterium tuberculosis* H37Rv strain by broth dilution assay method.

Antimycobacterial activity tested against *M. tuberculosis* indicated that compounds **4b** and **6g** exhibited twofold enhanced potency than parent compound **1** and the results indicate that some of them exhibited promising activities and they deserve more consideration as potential antitubercular agents.

Compound **3c**, **4b** and **6c** exhibited good or moderate antibacterial inhibition and compounds **3h** and **7c** showed excellent antifungal activity.

1. Introduction

Despite the availability of effective treatments, tuberculosis (TB) remains a major public health threat. Recent world health organization (WHO) reports show that TB is responsible for more than three million deaths annually worldwide [1]. The raise can be attributed to increase in emergence of drug-resistant strains of *Mycobacterium tuberculosis*, multi drug-resistant (MDR) TB and extensively drug-resistant (XDR) TB. For this reason it is critical to discover new drugs acting with different mechanism from those drugs used in current therapy [2,3].

One of the important strategies for the designing of effective antitubercular agents is to develop inhibitors of mycobacterial cell-wall biosynthesis. The cell-wall of mycobacteria consists of a wide array of complex fatty acids, such as mycocerosic acid, mycolic acid, arabinogalactans and peptidoglycans [4,5]. Earlier, several reports have appeared that azole class of compounds posses key property (liphophilicity) that influence the ability of drug to reach target by transmembrane diffusion and show promising activity against resistant tuberculosis by blocking the biosynthesis of lipids [6,7].

Clubbed 1,2,4-triazole and 1,3,4-oxadiazole are new classes of azole anti-mycobacterials, which are proved to be highly active both in vitro and in vivo [8,9]. In continuation of our research on clubbed triazolyl-thiazoles [10–14] and clubbed triazolyl-isopropylthiazole, in previous communication it was proved isopropylthiazole moiety on coupling with other heterocyclic rings provides novel biologically active compounds that could be explored as potent antimicrobial and antitubercular agents [15]. The present study illustrates the details of further structural modifications carried out on clubbed isopropylthiazole with 1,3,4-oxadiazoles and 1,2,4-triazoles, studies its cytotoxicity, antimicrobial (bacterial and fungal) and antitubercular activity against H37Rv strain. The synthetic route and the sequence of reactions are depicted in Schemes 1 and 2.

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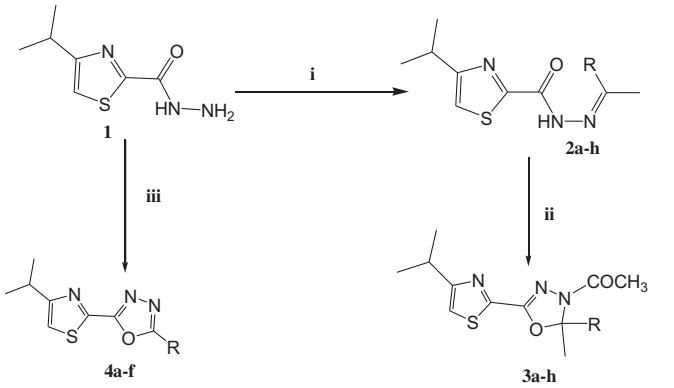


Scheme 1. Reagent and conditions: (i) CH_3OH/C_2H_5OH, $RCOCH_3$, reflux, 4–6 h; (ii) $(CH_3CO)_2O$, reflux, 4 h; (iii) $POCl_3$, RCOOH, reflux 5 h.

2. Chemistry

The reaction sequences employed for synthesis of target compounds are shown in Schemes 1 and 2, and their physical properties are depicted in Table 1.

The key intermediate in the present study 4-isopropylthiazole-2-carbahydrazide **1**, **2a–h** and **5** were prepared as per the literature [15]. Compounds **2a–h** on reacting with acetic anhydride afforded 1,3,4-oxadiazole derivatives **3a–h** in good yield. The 2-(4-isopropylthiazol-2-yl)-5-substituted-1,3,4-oxadiazoles **4a–f** were obtained on reacting compound **1** with appropriate aromatic acid in presence of phosphorus oxychloride (Scheme 1).

The triazole **5** was condensed with various substituted benzaldehydes in the presence of catalytic amount of concentrated sulphuric acid in refluxing ethanol to afford a series of 4-substituted-5-(4-isopropylthiazol-2-yl)-4H-1,2,4-triazole-3-thiols **6a–l**. Reaction of triazole **5** with appropriate heteroaromatic acids in the presence of phosphorous oxychloride produced a series of fused triazolo thiadiazoles **7a–f**. Compound **1** on reaction with phenylisothiocyanate in ethanol yielded compound 5-(4-isopropylthiazol-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol **8**. Further, alkylation of compound **8** was performed by the reaction with methyl iodide in basic media to obtain compound **9**.

3. Biological activity

The standard strains were procured from the American Type Culture Collection (ATCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The antibacterial activity of the synthesized compounds was performed by broth dilution method [16,17] against the following standard bacterial strains *Staphylococcus aureus* (ATCC 11632), *Streptococcus faecalis* (ATCC 14506), *Bacillus subtilis* (ATCC 60511), *Klebsiella pneumoniae* (ATCC 10031), *Escherichia coli* and *Pseudomonas aeruginosa* (ATCC 10145) and antifungal activity against yeasts: *Saccharomyces cerevisiae* (ATCC 9763, Sc) and *Candida tropicalis* (ATCC 1369, CT), mould: *Aspergillus niger* (ATCC 6275). MIC of compounds was determined against *M. tuberculosis* $H_{37}Rv$ strain by using broth dilution assay method. The cytotoxicity of the synthesized compounds was evaluated for their cytotoxic potential using A_{549} (lung adenocarcinoma) cell lines in the presence of fetal bovine serum.

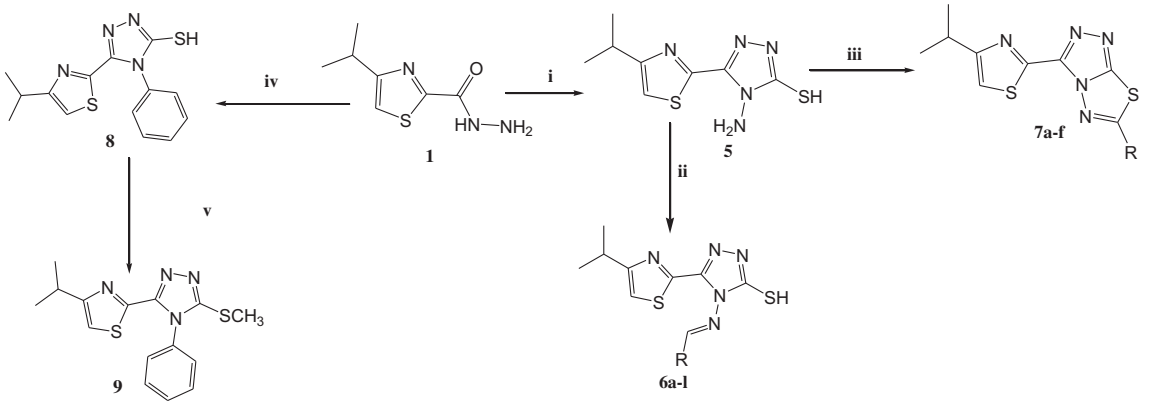
4. Results and discussion

The IR spectrum of compound **3d** revealed broad stretching band around 1713 cm^{-1} accounting for C=O amide carbonyl group. Lack of resonance's of NH and NH_2 and appearance of sharp singlet at δ 1.84 of CH_3 group in the ^1H NMR spectrum of compound **3d** accounted for oxadiazole ring formation and reconfirmed by the ^{13}C NMR peak at δ 67.58 due to C_2 of oxadiazole. The molecular ion peak *m/z* at 374.10 in mass spectrum of compound **3d** found to be in conformity with its molecular formula of the assigned structure.

The structure of **4b** was confirmed by lack of resonances corresponding to NH and NH_2 in the ^1H NMR spectrum and appearance of peaks at 154.34 and 165.71 due to C_2 and C_5 of oxadiazole in ^{13}C NMR spectra. The molecular ion base peak at *m/z* 305.04 confirmed structure of compound **4b**.

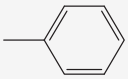
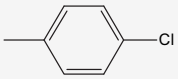
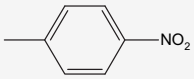
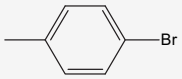
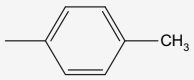
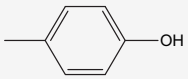
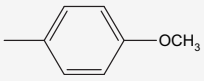
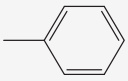
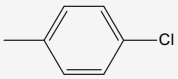
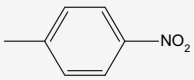
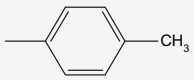
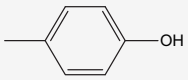
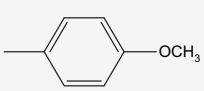
The absence of NH_2 stretch at 3248 cm^{-1} and appearance of C=N at 1674 cm^{-1} in IR spectrum of compound **6b** confirms the formation of Schiff base. In the ^1H NMR spectra of Schiff bases **6a–l**, a singlet corresponding to one proton characteristic of the N=CH group was observed in between δ 9.18–9.91 ppm and a singlet at 3.82 ppm integrating for 9 protons of 3 methoxyl groups substituted at 3,4,5-position of phenyl and a peak at δ 164.23 ppm due to HC=N in ^{13}C NMR spectra of Schiff base **6b** confirmed the formation of compound **6b**. Further, LC mass spectrum showed molecular ion peak at *m/z* 419.52 (100%) which is in agreement with the molecular formula $C_{18}H_{21}N_5O_3S_2$ of compound **6b**.

The absence of absorptions due to SH and NH_2 and the presence of a sharp absorption band at 1632 cm^{-1} due to secondary amino group in the IR spectrum of compounds **7a–f**, established formation of triazolo thiadiazoles. The ^1H NMR, ^{13}C NMR, mass spectra, and elemental analysis supported the structure of various synthesized triazolo thiadiazoles.



Scheme 2. Reagent and conditions: (i) CS_2, $NH_2NH_2 \cdot H_2O$, C_2H_5OH, KOH, reflux, 16 h; (ii) R–CHO, H_2SO_4, reflux 4 h (iii) R–COOH, $POCl_3$, reflux 5 h, (iv) PhNCS, C_2H_5OH, reflux 4 h (v) CH_3I, Na, C_2H_5OH, reflux 4 h.

Table 1
Analytical and physico-chemical data of synthesized compounds.

Compound	R	Molecular formula	M.W[a]	M.p (°C)[b]/crystallization solvent	Yield (%)	% Analysis of C, H, N found (calc.)[c]		
						C	H	N
3a	CH_3	$C_{12}H_{17}N_3O_2S$	267.35	126, Methanol	81	53.91 (54.01)	6.41 (6.23)	15.72 (15.65)
3b		$C_{17}H_{19}N_3O_2S$	329.42	116, Methanol	76	61.98 (61.45)	5.81 (5.86)	12.76 (12.72)
3c		$C_{17}H_{18}ClN_3O_2S$	363.86	121, Methanol	87	56.12 (56.45)	4.99 (4.49)	11.55 (11.09)
3d		$C_{17}H_{18}N_4O_4S$	374.41	128, Methanol	84	54.53 (56.23)	4.85 (4.65)	14.96 (14.09)
3e		$C_{17}H_{18}BrN_3O_2S$	408.31	136, Methanol	71	50.01 (50.65)	4.44 (4.60)	10.29 (10.21)
3f		$C_{18}H_{21}N_3O_2S$	343.44	116, Methanol	74	62.95 (62.76)	6.16 (6.69)	12.23 (12.62)
3g		$C_{17}H_{19}N_3O_3S$	345.42	124, Methanol	70	59.11 (59.87)	5.54 (5.87)	12.17 (12.65)
3h		$C_{18}H_{21}N_3O_3S$	359.44	123, Methanol	69	60.15 (60.82)	5.89 (5.87)	11.69 (11.65)
4a		$C_{14}H_{13}N_3OS$	271.34	231, Ethylacetate:hexane (2:4)	80	61.97 (61.88)	4.83 (4.88)	15.49 (15.09)
4b		$C_{14}H_{12}ClN_3OS$	305.78	223, Ethylacetate:hexane (2:4)	87	54.99 (54.09)	3.96 (3.76)	13.74 (13.87)
4c		$C_{14}H_{12}N_4O_3S$	316.34	198, Ethylacetate:hexane (2:4)	91	53.16 (53.65)	3.82 (3.76)	17.71 (17.38)
4d		$C_{15}H_{15}N_3OS$	285.36	196, Ethylacetate:hexane (2:4)	92	63.13 (63.48)	5.30 (5.39)	14.73 (14.38)
4e		$C_{14}H_{13}N_3O_2S$	287.34	242, Ethylacetate:hexane (2:4)	85	58.52 (58.27)	4.56 (4.45)	14.62 (14.65)
4f		$C_{15}H_{15}N_3O_2S$	301.36	232, Ethylacetate:hexane (2:4)	88	59.78 (58.39)	5.02 (5.09)	13.95 (13.86)

(*continued on next page*)

Table 1 (*continued*)

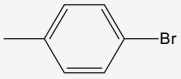
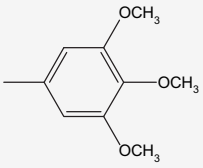
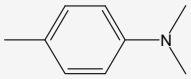
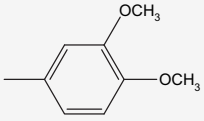
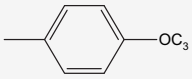
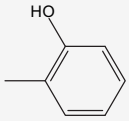
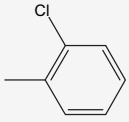
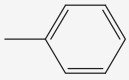
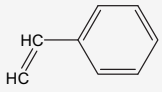
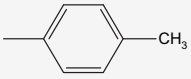
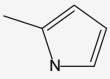
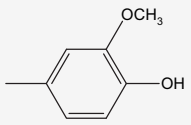
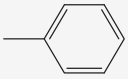
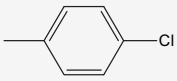
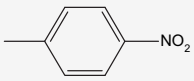
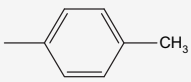
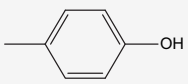
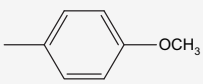
Compound	R	Molecular formula	M.W[a]	M.p (°C)[b]/crystallization solvent	Yield (%)	% Analysis of C, H, N found (calc.)[c]		
						C	H	N
6a		$C_{15}H_{14}BrN_5S_2$	408.34	160, Ethanol	69	44.12 (44.09)	3.46 (3.49)	17.15 (17.76)
6b		$C_{18}H_{21}N_5O_3S_2$	419.52	190, Ethanol	**86**	51.53 (51.87)	5.05 (5.38)	16.69 (16.47)
6c		$C_{17}H_{20}N_6S_2$	372.51	198, Ethanol	80	54.81 (54.87)	5.41 (5.47)	22.56 (22.38)
6d		$C_{17}H_{19}N_5O_2S_2$	389.5	206, Ethanol	75	52.42 (52.38)	4.92 (4.37)	17.98 (17.30)
6e		$C_{16}H_{17}N_5OS_2$	359.47	214, Ethanol	82	53.46 (53.45)	4.77 (4.77)	19.48 (19.45)
6f		$C_{15}H_{15}N_5OS_2$	345.44	240, Ethanol	81	52.15 (52.29)	4.38 (4.30)	20.27 (20.88)
6g		$C_{15}H_{14}ClN_5S_2$	363.89	170, Ethanol	86	49.51 (49.08)	3.88 (3.87)	19.25 (19.83)
6h		$C_{15}H_{15}N_5S_2$	329.44	156, Ethanol	79	54.69 (54.39)	4.59 (4.39)	21.26 (21.39)
6i		$C_{17}H_{17}N_5S_2$	355.09	186, Ethanol	76	57.44 (57.38)	4.82 (4.30)	19.70 (19.03)
6j		$C_{16}H_{17}N_5S_2$	343.47	240, Ethanol	88	55.95 (55.39)	4.99 (4.39)	20.39 (20.37)
6k		$C_{13}H_{14}N_6S_2$	318.42	232, Ethanol	83	49.04 (49.38)	4.43 (4.48)	26.39 (26.76)
6l		$C_{16}H_{17}N_5O_2S_2$	375.47	204, Ethanol	89	51.18 (51.16)	4.56 (4.38)	18.65 (18.23)

Table 1 (*continued*)

Compound	R	Molecular formula	M.W[a]	M.p (°C)[b]/crystallization solvent	Yield (%)	% Analysis of C, H, N found (calc.)[c]		
						C	H	N
7a		$C_{15}H_{13}N_5S_2$	327.43	245, Ethanol:DMF (2:1)	85	55.02 (55.39)	4.00 (4.39)	21.39 (21.87)
7b		$C_{15}H_{12}ClN_5S_2$	361.87	221, Ethanol:DMF (2:1)	77	49.79 (49.30)	3.34 (3.39)	19.35 (19.38)
7c		$C_{15}H_{12}N_6O_2S_2$	372.42	235, Ethanol:DMF (2:1)	69	48.38 (48.39)	3.25 (3.29)	22.57 (22.86)
7d		$C_{16}H_{15}N_5S_2$	341.45	245, Ethanol:DMF (2:1)	71	56.28 (56.73)	4.43 (4.39)	20.51 (20.43)
7e		$C_{15}H_{13}N_5OS_2$	343.43	219, Ethanol:DMF (2:1)	73	52.46 (52.38)	3.82 (3.38)	20.39 (20.01)
7f		$C_{16}H_{15}N_5OS_2$	357.45	243, Ethanol:DMF (2:1)	58	53.76 (53.06)	4.23 (4.37)	19.59 (19.99)
8	–	$C_{14}H_{14}N_4S_2$	302.42	211, Ethanol/water (1:1)	89	55.60 (55.11)	4.67 (4.98)	18.53 (18.38)
9	–	$C_{15}H_{16}N_4S_2$	316.44	234, Ethanol/water (1:1)	71	56.93 (56.01)	5.10 (5.62)	17.71 (17.72)

[a] Molecular weight of the compound.
[b] Melting point of the compound at their decomposition.
[c] Elemental analysis of C, H, and N were within ±0.4% of theoretical value.

The absence of signals due to NH and NH_2 group of hydrazide in ^{1}H NMR spectra of compound **8** and additional signals due to phenyl ring in the ^{1}H NMR and ^{13}C NMR spectra evidenced formation of triazole ring. The conversion of compound **8** to 3-(4-isopropylthiazol-2-yl)-5-(methylthio)-4-phenyl-4H-1,2,4-triazole compound **9** was confirmed by the appearance a singlet at δ 2.86 ppm in ^{1}H NMR spectra due to $-SCH_3$ group.

The results of antimicrobial testing of synthesized compounds against selected Gram-positive, Gram-negative bacteria, yeasts, moulds and *M. tuberculosis* $H_{37}Rv$ are illustrated in Tables 2 and 3 respectively.

The results of antimicrobial testing of synthesized compounds against selected Gram-positive, Gram-negative bacteria, yeasts, moulds and *M. tuberculosis* $H_{37}Rv$ are illustrated in Tables 2 and 3 respectively.

The antimicrobial activity of 1-(5-(4-isopropylthiazol-2-yl)-2, methyl 2-substituted-1,3,4-oxadiazol-3(2H)-yl)ethanones **3a–h** divulged that compound **3c** showed improved antimicrobial activity against tested bacterial and fungal species and excellent activity against *M. tuberculosis* $H_{37}Rv$ at MIC 8 μg/mL. Compounds **3f** and **3g** possessing *p*-CH_3 and *p*-OH substitution on phenyl ring exhibited moderate antimicrobial activity against gram +ve species and good activity against tested gram −ve species *K. pneumoniae* and *E. coli* than *P. aeruginosa*. The compound **3h** having *p*-methoxy substitution showed excellent antifungal activity at MIC 4–16 μg/mL than antibacterial action.

Evaluating the antimicrobial activity of the synthesized 2-(4-isopropylthiazol-2-yl)-5-substituted-1,3,4-oxadiazole derivatives **4a–f**, revealed compounds were more effective against the Gram-positive bacteria at MIC 8 to 32.5 μg/mL. Particularly, para-chloro substituted compound **4b** exhibited excellent inhibition at MIC 4–8 μg/mL against tested gram +ve bacteria and 4 μg/mL against *M. tuberculosis* $H_{37}Rv$. In contradiction compound **4f** comprising methoxy substitution showed moderate to good inhibition against tested Gram −ve organisms.

Schiff bases derived from 1,2,4-triazoles are reported to possess significant antimicrobial activity, particularly against *M. tuberculosis* $H_{37}Rv$ because of its increased ability to penetrate bacterial cell [18]. The antimicrobial activity of the series **6a–l** revealed that all the tested compounds possessed moderate to good inhibition, compounds **6g** and **6i** showed comparatively good activity against all tested microbial strains and excellent inhibition towards *M. tuberculosis* $H_{37}Rv$ at MIC 4 μg/mL. Compounds **6e** and **6k** possessing mono methoxy and pyrole substitution exhibit moderate activity at MIC 16–62.5 μg/mL.

The antimicrobial activity of the series 7**a–f** revealed compound **7b** comprising *p*-chloro substitution exhibited excellent inhibition, whereas the compound **7c** with higher electron withdrawing NO_2 showed increased antifungal inhibition, but loss of activity against tested bacterial species. The antimicrobial activity of compounds **8** and **9** exhibited good to moderate antimicrobial activity.

Compounds **3c**, **4c**, **6g** and **6i** were evaluated for their cytotoxic potential using A_{549} (lung adenocarcinoma) cell lines in the presence of fetal bovine serum. The compound **3c** showed maximum cytotoxicity at a concentration of 250 μM as depicted in Fig. 1. The other compounds showed appreciable cytotoxicity of about 50% of the vehicle control at a concentration of 250 μM.

Table 2
Antimicrobial activity expressed as MIC (μg/mL).

Compounds	Gram-positive organisms[a]			Gram-negative organisms[b]			Fungi[c]		
	Sa	Sf	Bs	Kp	Ec	Pa	Sc	Ct	An
3a	62.5	125	125	125	125	125	62.5	62.5	62.5
3b	31.25	31.25	62.5	62.5	62.5	31.25	125	31.25	62.5
3c	16	62.5	31.25	8	8	8	62.5	31.25	62.5
3d	31.25	62.5	62.5	62.5	16	31.25	31.25	62.5	31.25
3e	16	16	31.25	16	62.5	16	31.25	31.25	31.25
3f	125	125	16	31.25	16	125	16	31.25	31.25
3g	16	31.25	31.25	16	16	62.5	31.25	31.25	125
3h	31.25	125	31.25	125	125	125	4	8	8
4a	31.25	16	62.5	250	125	62.5	62.5	125	125
4b	8	8	4	125	125	125	62.5	125	62.5
4c	16	31.25	31.25	62.5	125	31.25	16	16	31.25
4d	31.25	16	31.25	125	62.5	125	62.5	125	31.25
4e	62.5	62.5	31.25	62.5	62.5	62.5	62.5	125	125
4f	31.25	31.25	31.25	31.25	16	16	31.25	31.25	31.25
6a	62.5	125	125	125	62.5	62.5	125	62.5	62.5
6b	125	62.5	125	62.5	62.5	125	62.5	125	62.5
6c	16	125	31.25	16	16	16	31.25	125	16
6d	125	125	125	125	125	250	125	125	250
6e	31.25	31.25	31.25	16	31.25	31.25	62.5	62.5	62.5
6f	250	250	250	250	250	250	125	125	125
6g	31.25	8	4	8	62.5	16	31.25	31.25	16
6h	250	31.25	31.25	31.25	62.5	31.25	125	62.5	62.5
6i	8	31.25	8	4	4	16	31.25	31.25	125
6j	31.25	125	31.25	125	125	31.25	31.25	31.25	16
6k	31.25	31.25	62.5	31.25	62.5	62.5	31.25	62.5	16
6l	250	125	125	125	125	125	250	125	125
7a	125	16	16	125	125	31.25	125	125	31.25
7b	8	16	8	16	16	8	31.25	31.25	125
7c	125	125	16	16	125	31.25	8	8	16
7d	125	125	62.5	250	125	62.5	250	125	125
7e	250	125	125	125	125	125	250	125	125
7f	31.25	16	125	31.25	125	31.25	16	16	31.25
8	16	125	16	31.25	16	31.25	8	16	16
9	16	16	31.25	31.25	62.5	16	16	16	31.25
Ciprofloxacin	≤5	≤5	≤1	≤1	≤1	□5	–	–	–
Norfloxacin	□5	□5	≤1	≤1	≤1	□5	–	–	–
Flucanozole	–	–	–	–	–	–	≤1	≤1	≤1

[a] The screening organisms. Gram-positive bacteria: *Staphylococcus aureus* (ATCC 11632, Sa), *Streptococus faecalis* (ATCC 14506, Sf), and *Bacillus subtilis* (ATCC 60511, Bs).
[b] the screening organisms. Gram-negative bacteria: *Klebsiella penumoniae* (ATCC 10031, Kp), *Escherichia coli* (ATCC 10536, Ec), and *Pseudomonas aeruginosa* (ATCC 10145, Pa).
[c] The screening organisms. Yeasts: *Saccharomyces cerevisiae* (ATCC 9763, Sc) and *Candida tropicalis* (ATCC 1369, Ct), mould: *Aspergillus niger* (ATCC 6275, An).

Table 3
Primary antitubercular activity.

Compound	MIC values (μg/mL) of *M. tuberculosis* $H_{37}Rv$
3a	125
3b	62.5
3c	16
3d	125
3e	125
3f	31.25
3g	62.5
3h	62.5
4a	31.25
4b	4
4c	62.5
4d	16
4e	125
4f	16
6a	125
6b	125
6c	125
6d	31.25
6e	31.25
6f	62.5
6g	4
6h	62.5
6i	4
6j	32.5
6k	31.25
6l	62.5
7a	62.5
7b	8
7c	31.25
7d	31.25
7e	62.5
7f	31.25
8	31.25
9	62.5
Isoniazid	0.25

5. Experimental

5.1. Chemical protocols

Melting points were determined in open capillary tubes in a Thomas Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on Shimadzu FT-IR 157, ^{1}H NMR and ^{13}C NMR spectra were recorded (in $CDCl_3$/DMSO-d_6) on a Bruker spectrometer at 300/400 MHz using TMS as an internal standard. Mass spectra (EI) on (AMD-604) mass spectrometer operating at 70 eV. Elemental analysis was performed on Thermo Finnigan Flash (EA 1112 CHNS Analyzer).

Thin layer chromatography (TLC) was performed through out the reaction on Merck silica gel GF_{254} aluminium sheets using mixture of different polar and nonpolar solvents in varying proportions and spots were observed using iodine as visualizing agent.

5.1.1. General procedure for the synthesis of 3-acetyl-5-(4-isopropylthiazole-4-yl)-2-methyl-2[1] substituted-2,3-dihydro-1,3,4-oxadiazoles (**3a**–**h**)

A mixture of compound (**2a**–**h**) (0.003 mol) and acetic anhydride (10 mL) was heated under reflux for 4 h. After the reaction mixture attained room temperature, excess acetic anhydride was decomposed by water and the mixture was stirred for further 30 min. The separated product was filtered, washed with water, dried and recrystallised.

*5.1.1.1. 1-(5-(4-Isopropylthiazol-2-yl)-2,2-dimethyl-1,3,4-oxadiazol-3(2H)-yl)ethanone (**3a**).* IR (KBr) ν max, cm^{-1}: 2987 (C–H of CH_3),1669 (amide C═O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.32 (s, 1H, thiazole-C_5), 3.26 (m, 1H, isopropyl), 2.21 (s, 3H of acetyl CH_3), 1.51 (s, 6H, $2CH_3$ of C_2 oxadiazole), 1.23 (d, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 170.11 (acetyl C═O), 162.98 (thiazole C_4), 154.29 (thiazole-C_2), 144.76 (oxadiazole C_5), 121.09 (thiazole-C_5), 67.43 (oxadiazole C_2), 33.12 (tertiary-1C-isopropyl), 27.24($2CH_3$ of C_2 oxadiazole), 24.16 (acetyl CH_3), 22.36 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 267.43 (M^+ 100.0%), 268.11 (M + 1, 13.6%), 269.10 (4.2%), 268.10 (1.1%), 269.11 (1.5%).

*5.1.1.2. 1-(5-(4-Isopropylthiazol-2-yl)-2-methyl-2-phenyl-1,3,4-oxadiazol-3(2H)-yl)ethanone (**3b**).* IR (KBr) ν max, cm^{-1}: 3056 (Ar C–H), 1632 (amide C═O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.6–7.8 (m, 5H, Ar–H), 7.21 (s, 1H, thiazole-C_5), 3.25 (m, 1H, isopropyl), 1.82 (s, oxadiazole-C_2-CH_3), 2.51 (s, 3H, acetyl CH_3), 1.25 (d, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 166.11 (acetyl C═O), 163.98 (thiazole C_4), 153.29 (thiazole-C_2), 142.76 (oxadiazole C_5), 136.76 (phenyl-C_1), 124.98 (phenyl-C_3 and C_5), 123.11 (phenyl-C_2 and C_6), 123.09 (thiazole-C_5), 122.11 (phenyl-C_4), 67.21 (oxadiazole C_2), 33.12 (tertiary-1C-isopropyl), 28.24 (CH_3 at oxadiazole C_2), 23.24 (acetyl CH_3), 21.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 329.12 (M^+ 100.0%), 330.12 (M + 1, 20.4%), 331.12 (5.3%), 331.13 (1.6%).

*5.1.1.3. 1-(2-(4-Chlorophenyl)-5-(4-isopropylthiazol-2-yl)-2-methyl-1,3,4-oxadiazol-3(2H)-yl)ethanone (**3c**).* IR (KBr) ν max, cm^{-1}: 3046 (Ar C–H), 1642 (amide C═O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.43–7.86 (m, 4H, Ar–H), 7.32 (s, 1H, thiazole-C_5), 3.36 (m, 1H, isopropyl), 2.23 (s, 3H of acetyl CH_3), 1.81 (s, oxadiazole-C_2-CH_3), 1.24 (d, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 167.11 (acetyl C═O), 162.98 (thiazole C_4), 152.29 (thiazole-C_2), 142.14 (oxadiazole C_5), 140.11 (phenyl-C_1), 132.11 (phenyl-C_4), 128.11 (phenyl-C_2 and C_6), 124.98 (phenyl-C_3 and C_5), 123.09 (thiazole-C_5), 65.11 (oxadiazole C_2), 33.43 (tertiary-1C-isopropyl), 28.53 (CH_3 at oxadiazole C_2), 23.81 (acetyl CH_3), 21.17 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 363.08 (M^+ 100.0%), 365.43 (36.4%), 365.09 (2.1%), 367.07 (1.1%).

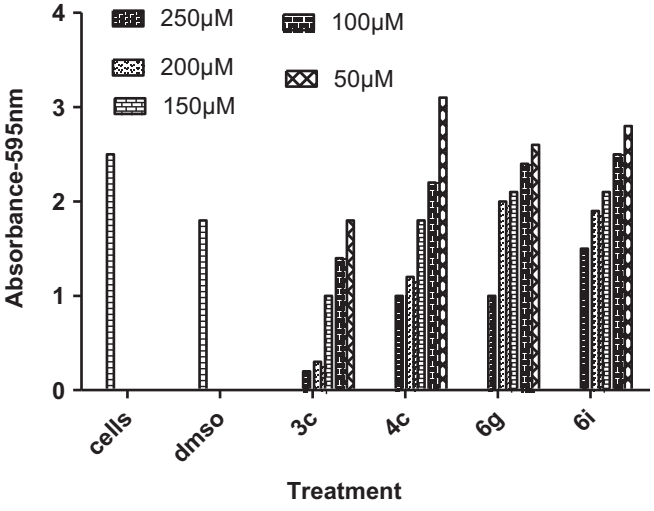


Fig. 1. Cytotoxic activity of compounds **3c**, **4c**, **6g** and **6i** tested in A_{549} cells by MTT assay. The bars reflect the viable cells in each treatment. Cells alone without any treatment, DMSO denote the vehicle control. The experiment was done in duplicate with triplicate readings of each experiment.

*5.1.1.4. 1-(5-(4-Isopropylthiazol-2-yl)-2-methyl-2-(4-nitrophenyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (**3d**).* IR (KBr) ν max, cm^{-1}: 3083 (Ar C–H), 1713 (amide C=O). ^1H NMR (DMSO-d_6, 300 MHz) δ: 7.53–7.80 (m, 4H, Ar–H), 7.32 (s, 1H, thiazole-C_5), 3.26 (m, 1H, isopropyl), 2.82 (s, 3H, acetyl CH_3), 1.84 (s, oxadiazole-C_2-CH_3), 1.21 (d, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 170.11 (acetyl C=O), 160.98 (thiazole C_4), 151.29 (thiazole-C_2), 144.76 (oxadiazole C_5), 140.87 (phenyl-C_1), 128.11 (phenyl-C_4), 125.98 (phenyl-C_3 and C_5), 123.11 (phenyl-C_2 and C_6), 111.09 (thiazole-C_5), 67.58 (oxadiazole C_2), 38.12 (tertiary-1C-isopropyl), 28.65 (oxadiazole-C_2-CH_3), 23.87 (terminal $2CH_3$-isopropyl), 23.41 (acetyl CH_3) ppm.

MS (%) 374.10 (M^+ 100.0%), 375.11 (M + 1, 18.7%), 376.10 (4.5%), 376.11 (2.9%), 375.10 (2.3%).

*5.1.1.5. 1-(2-(4-Bromophenyl)-5-(4-isopropylthiazol-2-yl)-2-methyl-1,3,4-oxadiazol-3(2H)-yl)ethanone (**3e**).* IR (KBr) ν max, cm^{-1}: 3036 (Ar C–H), 1640 (amide C=O). ^1H NMR (DMSO-d_6, 300 MHz) δ: 7.5–7.8 (m, 4H, Ar–H), 7.35 (s, 1H, thiazole-C_5), 3.26 (m, 1H, isopropyl), 2.41 (s, 3H of acetyl CH_3), 1.83 (s, oxadiazole-C_2-CH_3), 1.23 (d, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 169.11 (acetyl C=O), 161.21 (thiazole-C_4), 152.15 (thiazole-C_2), 144.21 (oxadiazole-C_5), 140.11 (phenyl-C_1), 129.11 (phenyl-C_2, C_6), 125.98 (phenyl-C_3 and C_5), 128.11 (phenyl-C_4), 113.21 (thiazole-C_5), 68.21 (oxadiazole C_2), 38.12 (tertiary-1C-isopropyl), 28.24 (oxadiazole-C_2-CH_3), 21.84 (terminal $2CH_3$-isopropyl), 23.11 (acetyl CH_3) ppm.

MS (%) 409.03 (M^+ 100.0%), 407.03 (97.5%), 410.03 (20.4%), 408.03 (19.9%), 411.02 (4.3%), 411.03 (2.4%), 409.04 (1.6%).

*5.1.1.6. 1-(5-(4-Isopropylthiazol-2-yl)-2-methyl-2-p-tolyl-1,3,4-oxadiazol-3(2H)-yl)ethanone (**3f**).* IR (KBr) ν max, cm^{-1}: 3096 (Ar C–H), 1636 (amide C=O). ^1H NMR (DMSO-d_6, 300 MHz) δ: 7.34 (s, 1H, thiazole-C_5), 7.3–7.8 (m, 4H, Ar–H), 3.24 (m, 1H, isopropyl), 2.10 (s, 3H, p-CH_3), 2.94 (s, 3H of acetyl CH_3), 1.87 (s, oxadiazole, C_2-CH_3), 1.23 (d, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 170.11 (acetyl C=O), 164.98 (thiazole C_4), 155.29 (thiazole-C_2), 145.32 (oxadiazole C_5), 139.14 (phenyl-C_1), 138.11 (phenyl-C_4), 127.98 (phenyl-C_3 and C_5), 126.11 (phenyl-C_2 and C_6), 113.09 (thiazole-C_5), 68.34 (oxadiazole C_2), 36.12 (tertiary-1C-isopropyl), 28.24 (oxadiazole-C_2-CH_3 CH_3), 23.24 (acetyl CH_3), 21.24 (terminal $2CH_3$-isopropyl), 20.60 (p-CH_3) ppm.

MS (%) 343.14 (M^+ 100.0%), 344.14 (M + 1, 19.8%), 345.13 (4.5%), 345.14 (2.6%), 344.13 (1.9%).

*5.1.1.7. 1-(2-(4-Hydroxyphenyl)-5-(4-isopropylthiazol-2-yl)-2-methyl-1,3,4-oxadiazol-3(2H)-yl)ethanone (**3g**).* IR (KBr) ν max, cm^{-1}: 3530 (OH), 3066 (Ar C–H), 1643 (amide C=O). ^1H NMR (DMSO-d_6, 300 MHz) δ: 7.45 (s, 1H, thiazole-C_5), 7.5–7.8 (m, 4H, Ar–H), 5.22 (s, 1H, OH), 3.24 (m, 1H, isopropyl), 2.31 (s, 3H of acetyl CH_3), 1.87 (s, oxadiazole-C_2-CH_3), 1.23 (d, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 169.61 (acetyl C=O), 166.43 (thiazole C_4), 158.11 (phenyl-C_4), 154.43 (thiazole-C_2), 144.09 (oxadiazole C_5), 139.65 (phenyl-C_1), 127.09 (phenyl-C_3 and C_5), 126.44 (phenyl-C_2 and C_6), 112.54 (thiazole-C_5), 68.09 (oxadiazole C_2), 36.18 (tertiary-1C-isopropyl), 28.24 (oxadiazole-C_2-CH_3), 23.09 (acetyl CH_3), 21.54 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 345.11 (M^+ 100.0%), 346.12 (M + 1, 18.7%), 347.11 (4.5%), 347.12 (2.6%), 346.11 (1.9%).

*5.1.1.8. 1-(5-(4-Isopropylthiazol-2-yl)-2-(4-methoxyphenyl)-2-methyl-1,3,4-oxadiazol-3(2H)-yl)ethanone (**3h**).* IR (KBr) ν max, cm^{-1}: 3056 (Ar C–H), 1673 (amide C=O). ^1H NMR (DMSO-d_6, 300 MHz) δ: 7.32 (s, 1H, thiazole-C_5), 7.6–7.9 (m, 4H, Ar–H), 3.74 (s, 3H, OCH_3), 3.26 (m, 1H, isopropyl), 2.13 (s, 3H, CH_3), 1.82 (s, oxadiazole-C_2-CH_3), 1.33 (d, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 170.11 (acetyl C=O), 163.65 (thiazole C_4), 157.54 (phenyl-C_4), 154.76 (thiazole-C_2), 143.76 (oxadiazole C_5), 139.82 (phenyl-C_1), 126.11 (phenyl-C_2 and C_6), 113.65 (phenyl-C_3 and C_5), 112.87 (thiazole-C_5), 68.65 (oxadiazole C_2), 53.24 (OCH_3), 36.12 (tertiary-1C-isopropyl), 29.11 (oxadiazole-C_2-CH_3), 24.54 (acetyl CH_3), 21.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 359.13 (M^+ 100.0%), 360.13 (M + 1, 21.5%), 361.13 (5.5%), 361.14 (1.9%).

*5.1.2. General procedure for synthesis of 2-(4-isopropylthiazol-2-yl)-5-substituted-1,3,4-oxadiazoles (**4a–f**)*

A mixture of compound **1** (0.001 M) and the appropriate aromatic acid (0.001 M) in phosphorus oxychloride (10 ml) was refluxed for 5 h. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus separated out was filtered, treated with dilute NaOH, washed with water and recrystallised.

*5.1.2.1. 2-(4-Isopropylthiazol-2-yl)-5-phenyl-1,3,4-oxadiazole (**4a**).* IR (KBr) ν max, cm^{-1}: 3057 (Ar C–H), 1651 (C=N). ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.01 (d, 2H, C_2 and C_6 phenyl), 7.74 (s, 1H, thiazole-C_5), 7.64 (d, 2H, C_3 and C_5 phenyl), 7.24 (m, 1H, C_4 phenyl), 3.21 (m, 1H, isopropyl), 1.24 (d, $J = 8.5$ Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.71 (oxadiazole-C_2), 161.95 (thiazole C_4), 154.54 (oxadiazole-C_5), 153.21 (thiazole-C_2), 131.76 (phenyl C_3 and C_5), 129.32 (C_4-phenyl), 127.51 (phenyl C_2 and C_6), 125.43 (C_1-phenyl), 115.32 (thiazole-C_5), 32.07 (tertiary-1C-isopropyl), 22.41 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 271.43 (M^+ 100.0%), 272.43 (M + 1, 16.1%), 273.81 (4.5%), 273.08 (1.6%), 272.07 (1.1%).

*5.1.2.2. 2-(4-Chlorophenyl)-5-(4-isopropylthiazol-2-yl)-1,3,4-oxadiazole (**4b**).* IR (KBr) ν max, cm^{-1}: 3091 (Ar C–H), 1636 (C=N). ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.26 (d, 2H, C_3 and C_5 phenyl), 7.85 (s, 1H, thiazole-C_5), 7.68 (d, 2H, C_2 and C_6 phenyl), 3.24 (m, 1H, isopropyl), 1.29 (d, $J = 8.5$ Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.71 (oxadiazole-C_2), 162.21 (thiazole-C_2), 160.35 (thiazole C_4), 154.34 (oxadiazole-C_5), 139.32 (C_4-phenyl), 134.81 (C_3 and C_5), 127.51 (C_2 and C_6), 125.43 (C_1-phenyl), 114.11 (thiazole-C_5), 32.65 (tertiary-1C-isopropyl), 21.41 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 305.2 (M^+ 100.0%), 307.04 (M + 1, 32.5%), 306.04 (17.1%), 308.04 (5.9%), 307.03 (4.5%), 309.03 (1.5%), 307.05 (1.1%).

*5.1.2.3. 2-(4-Isopropylthiazol-2-yl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (**4c**).* IR (KBr) ν max, cm^{-1}: 3061 (Ar C–H), 1646 (C=N). ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.06 (d, 2H, C_3 and C_5 phenyl), 7.88 (d, 2H, C_2 and C_6 phenyl), 7.75 (s, 1H, thiazole-C_5), 3.26 (m, 1H, isopropyl), 1.27 (d, $J = 8.5$ Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.21 (oxadiazole-C_2), 162.20 (thiazole-C_2), 159.48 (thiazole C_4), 154.43 (oxadiazole-C_5), 143.65 (C_4-phenyl), 135.76 (C_1-phenyl), 127.87 (phenyl C_2 and C_6), 118.48 (phenyl C_3 and C_5), 115.24 (thiazole-C_5), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 316.06 (M^+ 100.0%), 317.07 (M + 1, 15.4%), 318.06 (4.8%), 317.06 (2.3%), 318.07 (1.8%).

*5.1.2.4. 2-(4-Isopropylthiazol-2-yl)-5-p-tolyl-1,3,4-oxadiazole (**4d**).* IR (KBr) ν max, cm^{-1}: 3077 (Ar C–H), 1642 (C=N). ^1H NMR (DMSO-d_6, 300 MHz) δ: 7.87 (d, 2H, C_2 and C_6 phenyl), 7.61 (d, 2H, C_3 and C_5 phenyl), 7.45 (s, 1H, thiazole-C_5), 1.27 (d, $J = 8.5$ Hz, 6H, CH_3), 3.15 (m, 1H, isopropyl), 2.43 (s, 3H, CH_3 at C_4 phenyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 168.43 (thiazole-C_2), 165.87 (oxadiazole-C_2), 154.36 (oxadiazole-C_5), 144.43 (C_4-phenyl), 135.43 (C_1-phenyl), 127.34 (phenyl C_2 and C_6), 121.65 (thiazole C_4), 119.81 (phenyl C_3 and C_5), 115.24 (thiazole-C_5), 32.34 (tertiary-1C-isopropyl), 24.9 (CH_3 at C_4 phenyl), 21.47 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 285.09 (M^+ 100.0%), 286.10 (M + 1, 16.4%), 287.09 (4.7%), 286.09 (1.9%), 287.10 (1.6%).

*5.1.2.5. 2-(4-Isopropylthiazol-2-yl)-5-phenol-1,3,4-oxadiazole (**4e**).* IR (KBr) ν max, cm^{-1}: 3509 (OH), 3068 (Ar C–H), 1636 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.64 (d, 2H, C_2 and C_6 phenyl), 7.48 (d, 2H, C_3 and C_5 phenyl), 7.03 (s, 1H, thiazole-C_5), 5.08 (s, 1H, OH at C_4 phenyl), 3.21 (m, 1H, isopropyl), 1.43 (d, J = 8.5 Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.87 (oxadiazole-C_2), 163.19 (thiazole-C_2), 154.87 (oxadiazole-C_5), 151.21 (C_4-phenyl), 135.11 (C_1-phenyl), 125.11 (phenyl C_2 and C_6), 121.32 (thiazole C_4), 118.32 (phenyl C_3 and C_5), 115.15 (thiazole-C_5), 32.34 (tertiary-1C-isopropyl), 21.47 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 287.07 (M^+ 100.0%), 288.08 (M + 1, 15.4%), 289.43 (4.7%), 288.09 (1.9%), 289.08 (1.6%).

*5.1.2.6. 2-(4-Isopropylthiazol-2-yl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (**4f**).* IR (KBr) ν max, cm^{-1}: 3086 (Ar C-H), 1638 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.52 (d, 2H, C_2 and C_6 phenyl), 7.32 (s, 1H, thiazole-C_5), 7.16 (d, 2H, C_3 and C_5 phenyl), 3.75 (s, 3H, OCH_3 at C_4 phenyl), 3.45 (m, 1H, isopropyl), 1.22 (d, J = 8.5 Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.81 (thiazole-C_2), 162.41 (oxadiazole-C_2), 154.54 (oxadiazole-C_5), 144.43 (C_4-phenyl), 135.01 (C_1-phenyl), 127.92 (phenyl C_2 and C_6), 121.04 (thiazole C_4), 119.83 (phenyl C_3 and C_5), 115.73 (thiazole-C_5), 55.87 (OCH_3 at C_4 phenyl), 32.32 (tertiary-1C-isopropyl), 21.54 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 301.43 (M^+ 100.0%), 302.09 (M + 1, 18.4%), 303.72 (4.5%), 303.34 (1.3%).

*5.1.3. General method for synthesis of 4-substituted- 5-(4-isopropylthiazol-2-yl)-4H-1,2,4-triazole-3-thiols (**6a**–**l**)*

A mixture of compound **5** (10 mmol), substituted benzaldehydes (10 mmol) and 4–5 drops of concentrated sulphuric acid in ethanol was heated to reflux for 4 h. The resulting solution was cooled to room temperature and the precipitated solid was filtered under suction, washed with cold ethanol and recrystallized from hot ethanol.

*5.1.3.1. 4-(4-Bromobenzylideneamino)-5-(4-isopropylthiazol-2-yl)-4H-1,2,4-triazole-3-thiol (**6a**).* IR (KBr) ν max, cm^{-1}: 3357 (NH), 1654 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 14.49 (s, 1H, N=C-SH, disappeared on D_2O exchange), 10.63 (s, 1H, N=CH), 7.6–7.9 (4H, phenyl), 7.61 (s, 1H, thiazole-C_5), 3.15 (m, 1H, isopropyl), 1.23 (d, J = 8.5 Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 168.31 (C=S), 164.44 (HC=N), 162.21 (thiazole C_4), 153.14 (thiazole C_2), 142.51 (triazole-C_5), 125–132 (Ar C_1–C_6), 116.19 (thiazole-C_5), 30.123 (tertiary-1C-isopropyl), 23.12 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 407.99 (M^+ 100.0%), 408.99 (M + 1, 99.1%), 409.99 (17.5%), 410.98 (9.1%), 408.98 (9.1%), 409.98 (3.6%), 410.99 (1.9%), 407.98 (1.8%), 411.98 (1.7%).

*5.1.3.2. 4-(3,4,5-Trimethoxybenzylideneamino)-5-(4-isopropylthiazol-2-yl)-4H-1,2,4-triazole-3-thiol (**6b**).* IR (KBr) ν max, cm^{-1}: 3257 (NH), 1674 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 14.29 (s, 1H, N=C–SH, disappeared on D_2O exchange), 9.73 (s, 1H, N=CH), 7.11 (s, 1H, thiazole-C_5), 7.8–8.0 (2H, Ar H), 4.21 (s, 9H OCH_3), 3.15 (m, 1H, isopropyl), 1.23 (d, J = 8.5 Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 168.31 (C=S), 164.23 (HC=N), 162.21 (thiazole C_4), 152.15 (thiazole-C_2), 150.43 (Ar C_3–C_5), 143.53 (triazole-C_5), 142.27 (Ar C_6), 139.21 (Ar C_4), 132.27 (Ar C_1), 107 (Ar C_2–C_6), 116.19 (thiazole-C_5), 53.82 ($3OCH_3$), 30.123 (tertiary-1C-isopropyl), 23.12 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 419.11 (M^+ 100.0%), 420.11 (M + 1, 23.3%), 421.10 (9.1%), 422.11 (1.9%), 421.12 (1.9%), 421.11 (1.3%).

*5.1.3.3. 4-(4-Dimethylaminebenzylideneamino)-5-(4-isopropylthiazol-2-yl)-4H-1,2,4-triazole-3-thiol (**6c**).* IR (KBr) ν max, cm^{-1}: 3356 (NH), 1654 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 13.29 (s, 1H, N=C-SH, disappeared on D_2O exchange), 9.81 (s, 1H, N=CH), 7.8–8.0 (4H, ArH), 7.11 (s, 1H, thiazole-C_5), 3.15 (m, 1H, isopropyl), 3.0 (s, 6H, N-$2CH_3$), 1.23 (d, J = 8.5 Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 164.24 (C=S), 164.49 (HC=N), 162.21 (thiazole C_4), 152.27 (Ar C_4–), 150.15 (thiazole-C_2), 142.5 (triazole-C_5), 131.54 (Ar C_1), 129.76 (Ar C_3 C_5), 116.19 (thiazole-C_5), 115.09 (Ar C_2–C_6), 41.04 (N-$2CH_3$), 30.123 (tertiary-1C-isopropyl), 23.12 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 372.12 (M^+ 100.0%), 373.12 (M + 1, 22.2%), 374.11 (9.1%), 375.12 (1.7%), 374.13 (1.6%).

*5.1.3.4. 4-(3,4-Dimethoxybenzylideneamino)-5-(4-isopropylthiazol-2-yl)-4H-1,2,4-triazole-3-thiol (**6d**).* IR (KBr) ν max, cm^{-1}: 3266 (NH), 1655 (amide C=N). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 13.59 (s, 1H, N=C–SH, disappeared on D_2O exchange), 9.91 (s, 1H, N=CH), 7.6–8.0 (3H, ArH), 7.51 (s, 1H, thiazole-C_5), 3.82 (s, 6H, OCH_3), 3.65 (m, 1H, isopropyl), 1.23 (d, J = 8.5 Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 163.09 (HC=N), 161.87 (C=S), 160.98 (thiazole C_4), 151.29 (thiazole-C_2), 148.11 (phenyl-C_4), 146.11 (phenyl-C_5), 145.29 (triazole-C_5), 128.11 (phenyl-C_1), 125.98 (phenyl-C_3), 123.11 (phenyl-C_2, C_6), 111.09 (thiazole-C_5), 57.21 ($2OCH_3$), 33.12 (tertiary-1C-isopropyl), 22.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 389.10 (M^+ 100.0%), 390.10 (M + 1, 22.1%), 391.09 (9.1%), 391.10 (2.7%), 392.10 (1.8%).

*5.1.3.5. 4-(4-Methoxybenzylideneamino)-5-(4-isopropylthiazol-2-yl)-4H-1,2,4-triazole-3-thiol (**6e**).* IR (KBr) ν max, cm^{-1}: 3276 (NH), 1632 (amide C=N). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 14.09 (s, 1H, N=C–SH, disappeared on D_2O exchange), 9.19 (s, 1H, N=CH), 7.11 (s, 1H, thiazole-C_5), 7.6–8.0(4H, ArH), 3.62 (3H OCH_3), 3.15 (m, 1H, isopropyl), 1.23 (d, J = 8.5 Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.89 (HC=N), 163.98 (thiazole C_4), 160.43 (C=S), 158.11 (phenyl-C_4), 151.29 (thiazole-C_2), 147.29 (triazole-C_5), 128.54 (phenyl-C_1), 125.98 (phenyl-C_3 and C_5), 118.11 (phenyl-C_2, C_6), 111.09 (thiazole-C_5), 53.09 (OCH_3), 31.12 (tertiary-1C-isopropyl), 23.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 359.09 (M^+ 100.0%), 360.09 (M + 1, 19.1%), 361.08 (9.1%), 361.09 (2.2%), 360.08 (1.8%), 362.09 (1.6%).

*5.1.3.6. 4-(2-Hydroxybenzylideneamino)-5-(4-isopropylthiazol-2-yl)-4H-1,2,4-triazole-3-thiol (**6f**).* IR (KBr) ν max, cm^{-1}: 3550 (OH), 1643 (C=N), 3327 (NH). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 14.19 (s, 1H, N=C-SH), 1.23 (d, J = 8.5 Hz, 6H, CH_3), 9.44 (s, 1H, N=CH), 7.8–8.0 (4H,ArH), 7.11 (s, 1H, thiazole-C_5), 3.15 (m, 1H, isopropyl), 5.49 (s, 1H OH) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 167.54 (HC=N), 162.98 (thiazole C_4), 160.87 (C=S), 158.11 (phenyl-C_2), 151.29 (thiazole-C_2), 147.29 (triazole-C_5), 125.98 (phenyl-C_4 and C_5), 123.11 (phenyl-C_3, C_6), 118.11 (phenyl-C_1), 111.09 (thiazole-C_5), 31.12 (tertiary-1C-isopropyl), 23.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 345.07 (M^+ 100.0%), 346.08 (M + 1, 16.4%), 347.07 (9.6%), 346.07 (3.4%), 348.07 (1.6%), 347.08 (1.5%).

*5.1.3.7. 4-(2-Chlorobenzylideneamino)-5-(4-isopropylthiazol-2-yl)-4H-1,2,4-triazole-3-thiol (**6g**).* IR (KBr) ν max, cm^{-1}: 3347 (NH), 1654 (C=N). ^1H NMR (DMSO-d_6, 300 MHz) δ: 13.59 (s, 1H, N=C-SH, disappeared on D_2O exchange), 9.76 (s, 1H, N=CH), 7.11 (s, 1H, thiazole-C_5), 7.6–8.0(4H, ArH), 3.15 (m, 1H, isopropyl), 1.23 (d, $J = 8.5$ Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 167.09 (HC=N), 160.98 (thiazole C_4), 158.33 (C=S), 151.29 (thiazole-C_2), 147.29 (triazole-C_5), 128.11 (phenyl-C_4), 125.98 (phenyl-C_3 and C_5), 123.11 (phenyl-C_2, C_6), 111.09 (thiazole-C_5), 119.11 (phenyl-C_1), 38.12 (tertiary-1C-isopropyl), 21.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 363.04 (M^+ 100.0%), 365.03 (M + 1, 41.0%), 364.04 (18.0%), 366.04 (6.8%), 367.03 (3.2%), 364.03 (1.8%), 365.04 (1.8%), 366.03 (1.3%).

*5.1.3.8. 4-(Benzylideneamino)-5-(4-isopropylthiazol-2-yl)-4H-1,2,4-triazole-3-thiol (**6h**).* IR (KBr) ν max, cm^{-1}: 3383 (NH), 1641 (C=N). ^1H NMR (DMSO-d_6, 300 MHz) δ: 13.19 (s, 1H, N=C–SH, disappeared on D_2O exchange), 9.38 (s, 1H, N=CH), 7.11 (s, 1H, thiazole-C_5), 7.8–8.0 (5H, ArH), 3.15 (m, 1H, isopropyl), 1.23 (d, $J = 8.5$ Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 167.09 (HC=N), 162.65 (C=S), 160.98 (thiazole C_4), 151.29 (thiazole-C_2), 147.29 (triazole-C_5), 128.43 (phenyl-C_3, C_5), 127.29 (phenyl-C_4), 123.47 (phenyl-C_1), 121.34 (phenyl-C_2, C_6), 111.09 (thiazole-C_5), 38.12 (tertiary-1C-isopropyl), 23.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 329.08 (M^+ 100.0%), 330.08 (M + 1, 18.0%), 331.07 (9.1%), 330.07 (1.8%), 331.08 (1.8%), 332.08 (1.5%).

*5.1.3.9. 4-((E)-3-phenylallylideneamino)-5-(4-isopropylthiazol-2-yl)-4H-1,2,4-triazole-3-thiol (**6i**).* IR (KBr) ν max, cm^{-1}: 3216 (NH), 1634 (C=N). ^1H NMR (DMSO-d_6, 300 MHz) δ: 14.09 (s, 1H, N=C–SH, disappeared on D_2O exchange), 9.18 (s, 1H, N=CH), 7.11 (s, 1H, thiazole-C_5), 7.6–7.8(5H,ArH), 5.73–6.5 (2H, CH=CH,), 3.15 (m, 1H, isopropyl), 1.23 (d, $J = 8.5$ Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 167.12 (HC=N), 162.98 (thiazole C_4), 160.32 (C=S), 151.29 (thiazole-C_2), 145.29 (triazole-C_5), 139 (C=CH), 121.54 (phenyl-C_2, C_6), 127.09 (phenyl-C_4), 123.65 (phenyl-C_1), 124.76 (phenyl-C_3 and C_5), 119.73 (CH=CH), 111.09 (thiazole-C_5), 38.12 (tertiary-1C-isopropyl), 23.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 355.09 (M^+ 100.0%), 356.10 (M + 1, 18.6%), 357.09 (9.4%), 356.09 (3.4%), 358.09 (1.9%), 357.10 (1.9%).

*5.1.3.10. 4-(4-Methylbenzylideneamino)-5-(4-isopropylthiazol-2-yl)-4H-1,2,4-triazole-3-thiol (**6j**).* IR (KBr) ν max, cm^{-1}: 3266 (NH), 1652 (C=N). ^1H NMR (DMSO-d_6, 300 MHz) δ: 14.29 (s, 1H, N=C–SH, disappeared on D_2O exchange), 9.65 (s, 1H, N=CH), 7.8–8.0(4H, ArH), 7.11 (s, 1H, thiazole-C_5), 3.15 (m, 1H, isopropyl), 2.15 (S, 1H, CH_3), 1.23 (d, $J = 8.5$ Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 167.87 (HC=N), 163.43 (thiazole C_4), 160.09 (C=S), 151.29 (thiazole-C_2), 145.29 (triazole-C_5), 138.11 (phenyl-C_4), 126.98 (phenyl-C_2, C_6), 127.98 (phenyl-C_1), 124.32 (phenyl-C_3 and C_5), 111.09 (thiazole-C_5), 38.12 (tertiary-1C-isopropyl), 24.15 (CH_3), 23.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 343.09 (M^+ 100.0%), 344.10 (M + 1, 17.5%), 345.09 (9.4%), 344.09 (3.4%), 346.09 (1.8%), 345.10 (1.7%).

*5.1.3.11. 4-((2H-Pyrrol-2-yl)methyleneamino)-5-(4-isopropylthiazol-2-yl)-4H-1,2,4-triazole-3-thiol (**6k**).* IR (KBr) ν max, cm^{-1}: 3288 (NH), 1654 (C=N).

^1H NMR^1H NMR (DMSO-d_6, 300 MHz) δ: 14.29 (s, 1H, N=C–SH, disappeared on D_2O exchange), 12.29 (s, 1H, py NH), 9.23 (s, 1H, N=CH), 7.11 (s, 1H, thiazole-C_5), 6.1–7.25 (s 3H,py), 3.15 (m, 1H, isopropyl), 1.23 (d, $J = 8.5$ Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 167.12 (HC=N), 162.98 (thiazole C_4), 160.76 (C=S), 151.29 (thiazole-C_2), 145.09 (triazole-C_5), 128.98–118.11 (pyrroll-$C_5C_2C_3C_4$), 111.09 (thiazole-C_5), 30.12 (tertiary-1C-isopropyl), 23.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 318.07 (M^+ 100.0%), 319.08 (M + 1, 14.2%), 320.07 (9.6%), 319.07 (3.8%), 321.07 (1.4%).

*5.1.3.12. 4-(2-Hydroxy,4-methoxy benzylideneamino)-5-(4-isopropyl thiazol-2-yl)-4H-1,2,4-triazole-3-thiol (**6l**).* IR (KBr) ν max, cm^{-1}: 3540 (OH), 3256 (NH), 1652 (C=N). ^1H NMR (DMSO-d_6, 300 MHz) δ: 13.59 (s, 1H, N=C–SH), 9.78 (s, 1H, N=CH), 1.23 (d, $J = 8.5$ Hz, 6H, CH_3), 4.59 (s, 1H, OH), 3.59 (s, 1H, CH_3), 3.15 (m, 1H, isopropyl), 7.51 (s, 1H, thiazole-C_5), 7.6–7.75 (3H, ArH) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 167.94 (HC=N), 162.98 (thiazole C_4), 160.21 (C=S), 158.11 (phenyl-C_5), 151.29 (thiazole-C_2), 148.11 (phenyl-C_4), 145.18 (triazole-C_5), 124.21 (phenyl-C_1), 123.76 (phenyl-C_2, C_6), 119.43 (phenyl-C_3), 111.09 (thiazole-C_5), 48.11 (phenyl-OCH_3), 31.12 (tertiary-1C-isopropyl), 21.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 375.08 (M^+ 100.0%), 376.09 (M + 1, 17.6%), 377.08 (9.4%), 376.08 (3.4%), 377.09 (2.1%), 378.08 (1.8%).

*5.1.4. General method for the synthesis of 3-(4-isopropylthiazol-2-yl)-6-substituted-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (**7a–f**)*

An equimolar mixture (0.1 M) of compound **5** and appropriate aromatic acids in phosphorus oxychloride (10 mL) was refluxed for 5 h. The reaction mixture was cooled to room temperature and then gradually poured on to crushed ice with stirring. The mixture was allowed to stand overnight and the solid separated out was filtered, treated with dilute sodium hydroxide solution and washed thoroughly with cold water. The compound so obtained was dried and recrystallized.

*5.1.4.1. 3-(4-Isopropylthiazol-2-yl)-6-phenyl-[1,2,4]triazolo[3,4-b]-[1,3,4]thiadiazole (**7a**).* IR (KBr) ν max, cm^{-1}: 3091 (Ar C–H), 1636 (C=N). ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.02 (d, 2H, C_3 and C_5 phenyl), 7.74 (s, 1H, thiazole-C_5), 7.71 (t, 2H, C_2 and C_6 phenyl), 7.64 (m, 1H, C_4 phenyl), 3.21 (m, 1H, isopropyl), 1.24 (d, $J = 8.5$ Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 168.21 (thiadiazole-C_2), 161.95 (thiazole C_4), 154.58 (thiazole-C_2), 149.87 (triazole-C_2), 147.11 (triazole-C_5), 134.43 (C_1-phenyl), 131.32 (C_4-phenyl), 129.34 (C_2 and C_6), 124.76 (C_3 and C_5), 112.69 (thiazole-C_5), 33.38 (tertiary-1C-isopropyl), 23.71 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 327.43 (M^+ 100.0%), 328.09 (M + 1, 19.7%), 329.36 (9.7%), 330.00 (1.6%), 329.81 (1.3%).

*5.1.4.2. 3-(4-Isopropylthiazol-2-yl)-6-(4-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**7b**).* IR (KBr) ν max, cm^{-1}: 3081 (Ar C–H), 1629 (C=N). ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.12 (d, 2H, C_3 and C_5 phenyl), 7.61 (t, 2H, C_2 and C_6 phenyl), 7.45 (s, 1H, thiazole-C_5), 3.32 (m, 1H, isopropyl), 1.31 (d, $J = 8.5$ Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 166.01 (thiadiazole-C_2), 163.29 (triazole-C_5), 162.59 (thiazole C_4), 154.87 (thiazole-C_2), 150.54 (triazole-C_2), 131.11 (C_1-phenyl), 130.38 (C_4-phenyl), 128.37 (C_2 and C_6), 124.18 (C_3 and C_5), 112.6 (thiazole-C_5), 33.43 (tertiary-1C-isopropyl), 23.58 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 361.34 (M^+ 100.0%), 363.54 (M + 1, 41.3%), 362.36 (16.4%), 364.98 (8.0%), 362.02 (3.4%), 365.02 (3.3%), 363.03 (1.5%).

*5.1.4.3. 3-(4-Isopropylthiazol-2-yl)-6-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**7c**).* IR (KBr) ν max, cm^{-1}: 3086 (Ar C–H), 1626 (C=N). ^1H NMR (DMSO-d_6, 300 MHz) δ: 7.61 (d, 2H, C_3 and C_5 phenyl), 7.49 (t, 2H, C_2 and C_6 phenyl), 7.26 (s, 1H, thiazole-C_5), 3.38 (m, 1H, isopropyl), 1.54 (d, $J = 8.5$ Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 168.01 (thiadiazole-C_2), 161.43 (thiazole C_4), 154.17 (thiazole-C_2), 149.17 (triazole-C_2), 147.17 (triazole-C_5), 134.36 (C_1-phenyl), 131.11 (C_4-phenyl), 129.31 (phenyl C_2 and C_6), 124.32 (phenyl C_3 and C_5), 112.01 (thiazole-C_5), 33.16 (tertiary-1C-isopropyl), 23.98 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 372.05 (M^+ 100.0%), 373.05 (M + 1, 18.0%), 374.04 (9.1%), 374.05 (2.3%), 373.04 (2.2%), 375.05 (1.6%).

*5.1.4.4. 3-(4-Isopropylthiazol-2-yl)-6-p-tolyl-[1,2,4]triazolo[3,4-b]-[1,3,4]thiadiazole (**7d**).* IR (KBr) ν max, cm^{-1}: 3078 (Ar C–H), 1642 (C═N). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.83 (d, 2H, C_3 and C_5 phenyl), 7.61 (t, 2H, C_2 and C_6 phenyl), 7.10 (s, 1H, thiazole-C_5), 3.18 (m, 1H, isopropyl), 2.33 (s, 3H, CH_3 at C_4 phenyl), 1.57 (d, J = 8.5 Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 168.01 (thiadiazole-C_2), 161.36 (thiazole C_4), 154.87 (thiazole-C_2), 149.17 (triazole-C_2), 147.11 (triazole-C_5), 134.15 (C_4-phenyl), 131.34 (C_1-phenyl), 129.10 (phenyl C_2 and C_6), 124.21 (C_3 and C_5), 112.01 (thiazole-C_5), 33.98 (tertiary-1C-isopropyl), 25.23 (CH_3 at C_4 phenyl), 23.43 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 341.08 (M^+ 100.0%), 342.08 (M + 1, 19.1%), 343.07 (9.1%), 343.08 (2.0%), 342.07 (1.8%), 344.08 (1.6%).

*5.1.4.5. 4-(3-(4-Isopropylthiazol-2-yl)-[1,2,4]triazolo[3,4-b]-[1,3,4]thiadiazol-6-yl)phenol (**7e**).* IR (KBr) ν max, cm^{-1}: 3501 (OH), 3094 (Ar C–H), 1638 (C═N). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.91 (t, 2H, C_2 and C_6 phenyl), 7.81 (d, 2H, C_3 and C_5 phenyl), 7.10 (s, 1H, thiazole-C_5), 5.03 (s, 1H, OH at C_4 phenyl), 3.17 (m, 1H, isopropyl), 1.23 (d, J = 8.5 Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 168.30 (thiadiazole-C_2), 161.34 (thiazole C_4), 154.65 (thiazole-C_2), 149.50 (triazole-C_2), 147.41 (triazole-C_5), 134.21 (C_1-phenyl), 134.94 (C_4-phenyl), 129.14 (phenyl C_2 and C_6), 124.16 (phenyl C_3 and C_5), 112.01 (thiazole-C_5), 33.02 (tertiary-1C-isopropyl), 23.54 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 343.06 (M^+ 100.0%), 344.06 (M + 1, 18.0%), 345.05 (9.1%), 345.06 (2.0%), 344.05 (1.8%), 346.06 (1.6%).

*5.1.4.6. 3-(4-Isopropylthiazol-2-yl)-6-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**7f**).* IR (KBr) ν max, cm^{-1}: 3098 (Ar C–H), 1627 (C═N). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.81 (t, 2H, C_2 and C_6 phenyl), 7.34 (d, 2H, C_3 and C_5 phenyl), 7.00 (s, 1H, thiazole-C_5), 3.89 (s, 1H, OCH_3 at C_4 phenyl), 3.23 (m, 1H, isopropyl), 1.43 (d, J = 8.5 Hz, 6H, CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 168.19 (thiadiazole-C_2), 161.21 (thiazole C_4), 154.43 (thiazole-C_2), 149.34 (triazole-C_2), 147.41 (triazole-C_5), 134.13 (C_1-phenyl), 132.18 (C_4-phenyl), 129.91 (phenyl C_2 and C_6), 124.16 (phenyl C_3 and C_5), 112.98 (thiazole-C_5), 55.89 (s, 1H, OCH_3 at C_4 phenyl), 33.87 (tertiary-1C-isopropyl), 23.43 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 357.07 (M^+ 100.0%), 358.08 (M + 1, 17.5%), 359.07 (9.7%), 358.07 (3.4%), 360.07 (1.7%), 359.08 (1.7%).

*5.1.5. Synthesis of 5-(4-isopropylthiazol-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol (**8**)*

A mixture of compound 1 (10 mmol) and phenylisothiocyanate (15 mmol) was refluxed in ethanol for 4 h to give a white solid. The resulting solid (10 mmol) was dissolved in 2 N NaOH and refluxed for 3 h. The resulting solution was cooled to room temperature and acidified to pH 3–4 with 37% HCl to give a white solid. The solid formed was filtered, washed with water and recrystallized.

IR (KBr) ν max, cm^{-1}: 3096 (C–H of aromatic), 2766 (SH).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 14.49 (s, 1H, N═C-SH, disappeared on D_2O exchange), 7.32 (s, 1H, thiazole-C_5), 7.1–7.9 (m, 5H, phenyl), 3.26 (m, 1H, isopropyl), 1.23 (d, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 184.82 (C═S), 161.21 (thiazole C_4), 153.84 (thiazole C_2), 125–132 (ArC_1–C_6), 38.54 (tertiary-1C-isopropyl), 116.10 (thiazole-C_5), 23.43 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 302.07 (M^+ 100.0%), 303.43 (M + 1, 16.1%), 304.06 (9.4%), 304.07 (1.9%), 305.07 (1.4%).

*5.1.6. Synthesis of 3-(4-isopropylthiazol-2-yl)-5-methyl-4-phenyl-4H-1,2,4-triazole (**9**)*

To a solution of compound **8** (10 mmol) in absolute ethanol, 1 equiv of sodium was added and the mixture was stirred at room temperature for 30 min. Then, methyl iodide (20 mmol) was added and refluxed for 4 h. After evaporating the solvent under reduced pressure a solid appeared. The solid was recyrstallized from ethanol/water (1:1) to obtain target compound.

IR (KBr) ν max, cm^{-1}: 3086 (NH), 2754 (SCH_3).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.6–7.9 (m, 5H, phenyl), 7.32 (s, 1H, thiazole-C_5), 3.26 (m, 1H, isopropyl), 2.86 (s, triazole-C_5, S-CH_3 of triazole), 1.23 (d, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 160.33 (thiazole C_4), 156.41 (triazole-C_5), 153.43 (thiazole C_2), 125–132 (ArC_1–C_6), 116.82 (thiazole-C_5), 39.11 (tertiary-1C-isopropyl), 24.13 (terminal $2CH_3$-isopropyl), 14.36 (triazole-C_5 S–CH_3) ppm.

MS (%) 316.08 (M^+ 100.0%), 317.08 (M + 1, 19.3%), 318.08 (9.6%), 319.08 (1.6%), 318.09 (1.3%).

5.2. Biological protocol

5.2.1. Antimicrobial activity

The antimicrobial susceptibility testing was performed in vitro by broth microdilution method [19–21]. The MIC determination of the synthesized compounds was carried out in side-by-side comparison with ciprofloxacin and norfloxacin against Gram-positive bacteria (*S. aureus*, *S. faecalis*, *B. subtilis*) and Gram-negative (*K. penumoniae*, *E. coli*, *P. aeruginosa*). The antifungal activity was assayed against yeasts (*C. tropicalis*, *S. cerevisiae*) and moulds (*A. niger*). The minimal inhibitory concentrations (MIC, μg/mL) were defined as the lowest concentrations of compound that completely inhibited the growth of each strain. Test compounds (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL) then diluted in culture medium (Mueller–Hinton Broth for bacteria and Sabouraud Liquid Medium for fungi), further progressive dilutions to obtain final concentrations of 1, 2, 4, 8, 16, 31.25, 62.5, 125, 250 and 500 μg/mL. DMSO never exceeded 1% v/v. The tubes were inoculated with 10^5 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 °C for 24 h. The growth control consisting of media and media with DMSO at the same dilutions as used in the experiments was employed.

5.2.2. Antitubercular activity

The preliminary antitubercular screening for test compounds was obtained for *M. tuberculosis* $H_{37}Rv$, the MIC of each drug was determined by broth dilution assay [22,23] and is defined as the lowest concentration of drug, which inhibits ≤99% of bacterial population present at the beginning of the assay. A frozen culture in Middlebrook 7H9 broth supplemented with 10% albumin-dextrose-catalase and 0.2% glycerol was thawed and diluted in broth to 10^5 cfu mL^{-1} (colony forming unit/mL) dilutions. Each test compound was dissolved in DMSO and then diluted in broth twice at the desired concentration. The final concentration of DMSO in the assay medium was 1.3%. Each U-tube was then inoculated with 0.05 mL of standardized culture and then incubated at 37 °C for 21 days. The growth in the U-tubes was compared with visibility against positive control (without drug), negative control (without drug and inoculum) and with standard isoniazid.

5.2.3. MTT assay for cell viability

Toxicity of compounds **3c**, **4c**, **6g**, and **6i** in A_{549} cell lines in the presence of 10% and 0.2% FBS respectively, was determined using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide reduction assay [24,25]. The compounds were dissolved in DMSO at 10 mM concentration and stored at −20 °C. The dilutions were made in culture medium before treatment.

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